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The T-body approach: potential for cancer immunotherapy

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Introduction

The immune system, with its exquisite specificity and potent effector mechanisms, has been suggested as an ideal tool for tumor therapy. Both the humoral and cell-mediated arms of the immune system have the ability to recognize foreign or tumor antigens via surface immunoglobulin or T cell receptor (TCR) binding. Moreover, both have the ability to eliminate the target cell by antibody recruitment of complement-mediated cytotoxicity or antibody-dependent cell-mediated cytotoxicity on the one hand, or by T cell-mediated cytolysis on the other. Nevertheless, neither humoral immunity nor cell-mediated immune responses have proved to be sufficiently effective in tumor therapy.

Cancer patients generally do not mount an effective immune response against their own tumors. Most spontaneous tumors escape the immune system because they are not sufficiently immunogenic or antigenic. Some, while potentially immunogenic, are able to evade or suppress a specific host-mediated immune response. Thus, most immunotherapeutic approaches attempt to provide the patient with either active or passive immunity directed against a particular tumor antigen. In this article we shall review a new approach, which we have recently developed, combining the humoral and cellular arms of the immune system to convey passive immunity against tumor cells. This technology (which we have named "T-bodies") makes use of cytotoxic immune effector cells transfected with a chimeric receptor of antibody-type specificity. These cells maintain their effector function, which is redirected to tumor cells by a receptor that recognizes antigen in the absence of MHC determinants.

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Our rationale in developing this technology stemmed from the many experimental and clinical studies which demonstrated difficulties in conventional immunotherapy. While anti-tumor antibodies, used alone or linked to a toxin, drug, or radioisotope, are effective against some tumors [26], antibodies penetrate large tumors poorly [18] and are therefore ineffective against most solid tumors, especially in advanced disease. Cytotoxic T cells are better able to home to and target a tumor site, are quite effective in penetrating tumors, and their cytotoxic effector mechanism is highly efficient and specific. Unfortunately, tumor-specific T cells are rare, and have been found only in several melanoma and renal cell carcinoma patients.

Our group [8, 9, 11–13] and others [3, 10, 19] have combined the humoral and cellular arms of the immune system to create the T-body approach. Using recombinant DNA technology, an antibody-derived variable (V) region of the desired specificity is grafted onto the TCR constant regions or onto a T cell signalling molecule of choice. This construct is then introduced into an effector (cytotoxic) cell population, thereby redirecting these cells to an antibody-determined specificity. Since the recognition element is derived from an antibody variable region, the redirected T cells are MHC independent and are not individual specific. We will describe here the basic construction and possible configurations of T-bodies, review results from some of our early studies using anti-hapten systems, and outline the progress that has been made towards adapting this approach to tumor immunotherapy.

The T-body concept: basic configurations

In our early studies [11–13, 20], we used a two-chain configuration (Fig. 1) which required the transfection of two constructs into the effector T cell of choice. In this configuration, the constant (C) region of each of the two (α and β) chains of the TCR is linked to an immunoglobulin heavy or light chain variable region (V_H or V_L) derived from an antibody of the desired specificity. The resulting chimeric receptor genes containing $C\alpha V_L$ and $C\beta V_H$, (or $C\alpha V_H$ and $C\beta V_L$) are then transfected into the effector T cell population. For surface expression and signal transduction the chimeric TCR (cTCR) heterodimers must associate with the CD3 complex. All the early studies with two-chain receptors utilized anti-hapten antibodies [3, 10–12, 19]. We transduced cytotoxic T cell hybridoma lines with trinitrophenyl (TNP)-specific chimeric receptor genes and showed specific secretion of interleukin-2 (IL-2) and cytolysis of hapten-modified target cells in response to antigen. Besides establishing the feasibility of the T-body approach, these experiments with hapten-specific receptors allowed the definition of some of the properties of chimeric TCR of this sort. Studies with haptens [13] showed that both V_H and V_L can be combined with either $C\alpha$ or $C\beta$ of the TCR. A single chimeric chain can associate with the complementary endogenous TCR to form a heterodimeric receptor. For many anti-hapten antibodies, most of the binding energy is contributed by the V_H chain. In such cases, a chimeric receptor chain containing the V_H can pair with the endogenous complementary TCR chain to yield a functional receptor with anti-hapten specificity. The cTCR could endow the T cell with non-MHC-restricted or -dependent antibody-type specificity, and in fact serves as the formal proof that MHC restriction is confined to the V region of the TCR. Transgenic mice have also been produced containing a $V_HC\alpha$ chimeric receptor chain [3]. This receptor associates in vivo with the native TCRβ chain, producing a functional receptor.

The use of antibody-derived V regions reactive with well-defined haptens enabled the biophysical parameters of T-body responses to be investigated. In the anti-TNP system, we showed that T cell lines expressing anti-TNP chimeric receptor genes were able to secrete IL-2 in response to TNP-modified target cells, or TNP-coupled proteins presented on a solid substrate, without MHC restriction. In addition, when the chimeric receptor was transfected into an effector cell capable of T cell-mediated cytolysis, these cells were able to kill TNP-modified targets. However, soluble antigen or hapten blocked the response, even if presented in polymeric form [e.g., as TNP-bovine serum albumin (BSA)].

Other studies showed variable effects of soluble antigen. Becker et al. [3] generated transgenic mice bearing T cells with chimeric alpha chains of anti-digoxin specificity. T cells derived from these mice could only be stimulated on a plastic substrate precoated with digoxin-modified BSA. In T cells expressing an anti-phosphocholine cTCR, it was shown that, while soluble antigen was not stimulatory, it did not inhibit the reactivity of T cells with immobilized antigen [10]. The differences observed are probably due to differences in the affinity or avidity of the various antibodies used to generate the chimeric receptors.

Having established the parameters of the T-body system with hapten antigens, we next extended it to more complex protein antigens, which may be of therapeutic value. For protein antigens, the V_H region, in most cases, is not sufficient to establish receptor specificity. For these antigens, two chimeric receptor chains (one with the V_H and the other with the V_L) must be introduced by either co-transfection or by two successive transfection steps, each with its own selectable marker. While the process is somewhat expedited by the use of a single expression vector containing both chimeric receptor subunits [20], the two-chain approach still has several drawbacks. The double transfection reduces transfection frequency, the chains are often expressed in unequal amounts, and they can form non-productive associations with endogenous TCR chains.

To further expedite the production of T-bodies, especially those directed against more complex tumor-associated antigens, we developed a single-chain receptor approach (Fig. 1). This was based on two observations. First, it was shown that many antibody V regions could be expressed as what has become known as a single-chain Fv (scFv). This is a construct incorporating heavy and light chain-derived V regions connected by a peptide linker [4, 15]. Thus, an antibody-derived binding specificity could be expressed on a single-chain structure. At about the same time, it was shown that any of several receptor subunits can serve as lymphocyte signalling molecules and can directly stimulate T cells, bypassing the requirement for a conventional TCR. These molecules include the γ and ζ chains of the FcR and TCR CD3 complex, respectively [24, 25].Combining these observations, we produced an scFvR – a single-chain chimeric receptor containing an antibody-derived recognition element in the form of an scFv, connected to the signalling subunit of an activation molecule of choice [8]. A spacer or hinge-like region added between the signalling and recognition moieties increases expression of the construct, and in many cases appears to be required for antigen recognition [22, 23]. The CD8 hinge [7] is often used for this purpose, and is thought to increase flexibility and exposure of the antigen-binding domain. We have found that the human IgG1 hinge region can also serve as an efficient spacer for certain configurations.

scFv receptors are expressed on the cell surface as homodimers (Fig. 1), or as heterodimers with endogenous signalling chains, and can trigger the activation of T cell hybridomas, T cell lines, and natural killer (NK) lines for lymphokine produc-

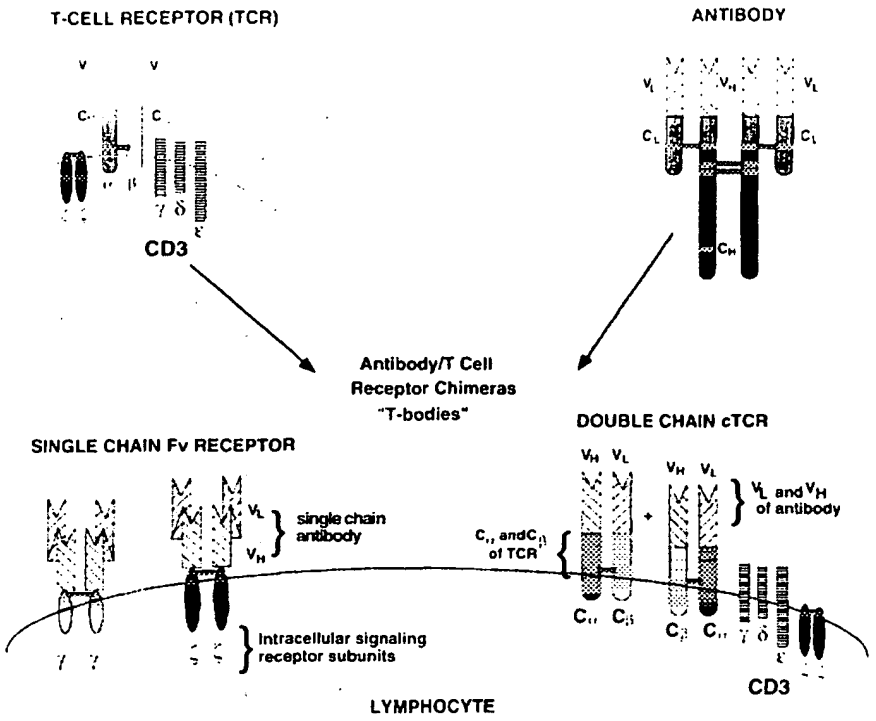

Fig. 1. Scheme depicting T-body configurations. Antibody variable regions (*top right*) in either a double-chain or single-chain configuration are spliced onto T cell receptor (*TCR*) or FcR signalling molecules (ζ or γ chains, respectively). The resulting chimeric genes are transfected into effector lymphocyte populations and expressed on the cell surface. The cTCR (*bottom right*) requires the CO3 complex for signal transduction, while the scFvR (*bottom left*) does not

tion and target cell killing, obeying the same rules observed for the double-chain cTCR described above [5, 8]. The single-chain receptor configuration confers several advantages to the T-body approach. Unlike the double-chain receptor, the scFvR is not dependent on the CD3 complex for surface expression, and can independently transduce signals for T cell stimulation. This design increases the number of possible signalling molecules that may be incorporated, thereby expanding the spectrum of cell types that can be activated using chimeric receptors. Moreover, because of the low efficiency of transgene expression in transfected T cells, the single-chain, single-transfection configuration is the only approach suitable for practical application.

Studies using chimeric receptor genes introduced into transgenic mice [6] have shown that stimulation through the chimeric scFv-ζ chain is insufficient to activate resting T cells. However, following conventional preactivation through the endogenous TCR complex, these cells may be triggered to respond by stimulation through the chimeric receptor. This is unlikely to pose a problem for the use of ζ-based constructs in tumor therapy, as the transfected effector cells are usually populations of preactivated T or NK cells.

Most recently, we have shown that T-bodies can be made that link an antibody-derived recognition element to an early signalling kinase of the T cell activation pathway, thereby bypassing entirely the need for receptor-derived chains. For T-body

immunotherapy, one advantage of this design is that it may improve activation of the effector cell and increase the fraction of cells stimulated. Chimeric receptors have been constructed containing an antibody-derived scFv region connected via a transmembrane and hinge region to the Syk protein tyrosine kinase (C. Fitzer-Attas, Z. Eshhar, unpublished data). These constructs are able to mediate a T cell activation signal resulting in IL-2 production when presented with antigen immobilized on a solid substrate, and both IL-2 production and target cell lysis when presented with target cells.

Methodological considerations

The basic methodologies for producing T-bodies are similar for all the different configurations. We have produced cassette vectors enabling the introduction of a scFv antibody recognition element upstream of a triggering molecule of choice. The scFv is cloned from a hybridoma cell producing a monoclonal antibody of the desired specificity by the polymerase chain reaction, using primers that we have previously described [9]. These primers also form the polypeptide linker that will connect the two V region domains. Another source of scFv may be those identified and selected from phage display libraries [31]. The signalling moiety is inserted directly behind the recognition element, or following a hinge or spacer region usually derived from the CD4 or CD8 T cell accessory molecules [7, 23].

The most substantial remaining technical problems in the production of T-bodies are the inefficiency of the transfection step and the long period in tissue culture required to obtain a population of cells stably expressing the novel receptor. These problems are especially critical when T-bodies are produced for therapeutic use. For use in therapy, T cells or NK cells from peripheral blood must be transfected with the chimeric T cell receptor of choice. Such in vitro-activated primary cells are often difficult to transfect, with only a small proportion of cells expressing the introduced gene, and change their in vivo homing properties if maintained in culture for extended periods. Retroviral vectors, which are able to infect dividing cells and introduce the foreign gene into a much higher proportion of cells, offer a partial solution to the problems of expression in primary cells [21]. The ideal vector would introduce stable expression of the novel chimeric receptor gene at a defined locus in the genome, in a high proportion of cells. This would eliminate the need for screening, selection, or enrichment steps, and thereby reduce the prolonged culture periods which often result in loss of the effector functions of the transduced lymphocytes. Furthermore, a defined integration site would eliminate the potential hazard of genetic transformation of the transfected cell.

Once a suitable transfection system is developed, several different cell types could be transduced. While most of our work has used murine and human T cell lines, NK cells are also suitable for the T-body approach. We [1] and others [30] have shown that human NK cell lines can be transfected with γ-chain-expressing chimeric receptors. The γ chain functions as a signalling molecule in these cells, redirecting their specificity towards the antigen of choice.

The inefficient and time-consuming process of transfection and selection of clones expressing the chimeric receptor gene also makes it difficult to evaluate large panels of similar receptor constructs and select the ones that will work best for a particular application. In an attempt to solve this problem, we are developing a transient assay

system, that will allow efficient transient expression of chimeric receptors, while coupling receptor activation to a simple and reliable reporter function. This will enable comparative studies to determine the relative efficiency of antibody V regions with different specificities or affinities, or of different spacer (hinge) and signalling domains for T cell activation. The most efficient constructs in transient assays can then be chosen to generate stable transfectants able to produce IL-2 and kill target cells.

As another system to test constructs with novel specificities, we have shown that γ-chain-containing chimeric receptors can be functionally expressed in the RBL-3H3 mast cell line [2]. Triggering with the appropriate antigen causes mast cell degranulation. This provides a convenient readout, as mast cell degranulation is easily assayed, and may be triggered by the cross-linking of a very small number of receptors. The testing of chimeric receptors on mast cells is also interesting because, unlike T cells, mast cells may be triggered by soluble antigen, so long as it is present in polyvalent or cross-linked form.

Model systems for T-bodies in therapy

Chimeric TCR with anti-hapten specificity are very sensitive to inhibition by monomeric or soluble antigen present in the culture medium. These results have implications for the use of chimeric TCR in tumor therapy, as they suggest that tumor antigens that are also present in the circulation will not be suitable as a target antigen for T-body-based therapy. Indeed, we have recently demonstrated this phenomenon using the 38C13 B cell lymphoma cell line [14]. A chimeric TCR was produced using the V region derived from an anti-idiotypic antibody specific for the surface immunoglobulin expressed on this lymphoma cell line. T cell lines transfected with this chimeric receptor are able to form rosettes and produce IL-2 following incubation with fixed 38C13 lymphoma cells. However, when live, unfixed target cells are used, no response is seen. We have shown that this is due to the blocking of the response by small amounts of 38C13 immunoglobulin secreted from the target cell.

In another study [20], T-bodies were made using an anti-IgE antibody (84.1C), which does not recognize IgE bound to the mast cell surface. This antibody does bind to IgE expressed on the surface of B cells which are destined to secrete IgE. We showed, in tissue culture, that cytotoxic T cell hybridomas transfected with a T-body receptor of anti-IgE specificity were able to specifically eliminate B cells expressing surface IgE, resulting in the complete elimination of IgE production and leaving IgG production unaffected. This system is a good model for tumor therapy because it involves the specific elimination of a subset of cells (in this case, surface IgE-expressing cells), without damage to the surrounding population.

The use of the T-body approach for tumor therapy

The use of scFv antibody fragments enables expression of antibody specificity with virtually no loss of affinity, in combination with a single-receptor chain and in a single-transfection step. The availability of this technology has enabled the development of several chimeric TCR that can serve as model systems for the eventual use of the T-body approach in tumor therapy. The cytotoxic potential of cells expressing such chimeric genes in various in vitro and in vivo model systems has been shown. All

these receptors used the scFv configuration for the antibody-based recognition element and the γ or ζ chain as a signalling moiety. In the first series of experiments, we made chimeric receptors using an scFv reactive with the Neu/HER-2 growth factor receptor, overexpressed on many breast and ovarian carcinomas. Cytotoxic T cell hybridomas [1, 29] expressing chimeric receptors with anti-Neu/HER2 specificity and the human γ signalling chain were able to secrete IL-2 in response to purified HER-2 antigen immobilized on plastic. Furthermore, transfected T cell hybridomas and and NK-like cell lines were able to specifically lyze tumor cell lines expressing the HER-2 antigen [1, 29].

The same specificity was utilized by Moritz et al. [22], who used a construct composed of an anti-HER2 monoclonal antibody-derived scFv, a hinge region derived from the CD4 or CD8 accessory molecules, and the mouse ζ chain. Cytotoxic T cells expressing this construct were able to specifically lyze HER-2 expressing target cell lines. This study was also extended to in vivo experiments; the growth of HER-2-expressing tumor cells in nude mice was slowed by adoptively transferred T cells expressing the chimeric receptor. Importantly, T cells bearing the chimeric receptors were shown to home to tumor sites [22].

To be useful in tumor therapy, a chimeric receptor must be able to redirect the specificity of primary T cells derived from the patient. Tumor-infiltrating lymphocytes (TIL), isolated from tumors and expanded in vitro in the presence of IL-2, are able to lyze autologous tumor cells [28]. This technique has been used with some success in the therapy of certain human cancers, where TIL can be found. Together with our collaborators at the Surgery Branch of the National Cancer Institute (USA), we endowed human TIL with anti-tumor specificity by transduction with a chimeric TCR gene [16]. TIL, derived from melanoma biopsies, were transfected, using retroviral transduction, with a chimeric receptor specific for an ovarian cancer-associated antigen [scFv derived from a monoclonal antibody reactive with the folate-binding protein (FBP)]. These cells were able to secrete granulocyte-macrophage colony-stimulating-factor in response to antigen-bearing cells and to specifically lyze the human ovarian carcinoma-derived IGROV-1 cell line, which expresses the FBP antigen [16]. The ability of the scFvR-expressing effector cells to secrete cytokines upon antigenic stimulation with tumor cells increases the potential of this approach in tumor therapy. The cytokines could stimulate an inflammatory response, which would have a tumoricidal or growth-inhibitory effect, not only on antigen-expressing tumor cells, but also on their antigen loss variants, which are not directly recognized by the T-body receptor.

To extend the list of cell types able to be redirected to anti-tumor specificity, the anti-HER2 and anti-FBP constructs were transfected into the YTS human NK-like cell line [1]. The γ receptor chain (and to a lesser degree the ζ chain) was able to convey signals, resulting in the specific lysis of FBP- or HER-2 expressing target cell lines.

The anti-FBP chimeric receptor was also tested for its ability to mediate an anti-tumor response in an in vivo system [17]. In a heterologous human model, IGROV-1 cells, which overexpress FBP, were injected into nude mice. The mice were then treated with murine TIL that had been transduced with the anti-FBP chimeric receptor gene. Nude mice injected with the human ovarian carcinoma cell line and treated with chimeric receptor-expressing TIL had significantly increased survival when compared with mice treated with control TIL or with saline. In another model, murine sarcoma cells expressing the human FBP were injected i.v. into syngeneic mice. After 3 days,

the mice were treated with murine TIL, expressing various chimeric receptors. When analyzed 3 weeks later, only the group receiving the FBP-specific TIL plus IL-2 demonstrated a dramatic reduction in lung metastasis [17]. These experiments begin to define the types of protocols that could be used in the eventual treatment of human tumors.

Towards therapy of human tumors

A schematic depiction of a protocol that might be used for treatment of human cancer with the T-body approach is shown in Fig. 2. According to such a protocol, tumor biopsies would first be screened for their reactivity with a panel of mAbs, for which chimeric scFvR are available. The specificity chosen should bind to the majority if not all of the malignant cells. Peripheral blood lymphocytes will be taken from the patient, stimulated in vitro using anti-CD3 antibodies, and then with IL-2 to induce selective activation and proliferation of T cells. These cells will then be transduced with previously prepared chimeric receptor retrovectors, selected for the particular patient's tumor. Depending on the transduction efficiency, receptor-positive transfectants can be enriched by selection for drug resistance, or directly for expression of the chimeric transgene using fluorescence-activated cell sorting, or panning with anti-idotypic antibodies. Redirected T cells will be propagated briefly in vitro and then reinfused into the patient.

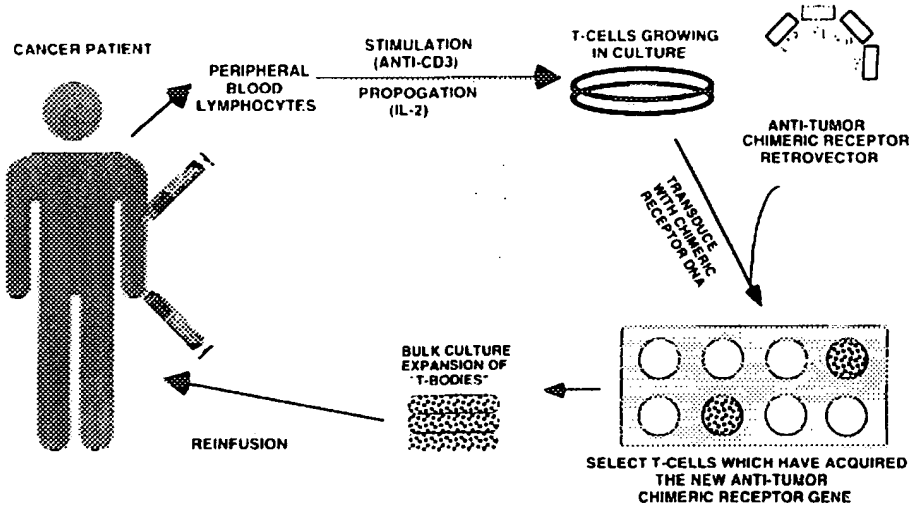

Fig. 2. The use of T-bodies in tumor therapy. In the approach we envisage tumor cells would first be screened for their reactivity with a panel of anti-tumor antigen antibodies for which T-body constructs are available (not shown). Following identification of appropriate target antigens for therapy, peripheral blood lymphocytes would be obtained from the patient, and T cells activated, propagated, and transfected (using retroviral transduction) with the appropriate chimeric receptor gene. Cells expressing the transgene would be selected, then expanded and reinfused into the patient, where they are expected to accumulate in tumors and either specifically lyze tumor cells or undergo stimulation to produce inflammatory cytokines. *IL-2*, Interleukin-2

We expect the redirected effector cells to reach the tumor site, undergo activation by the specific antigen, and either directly kill the tumor cells and/or secrete inflammatory cytokines which should attract a second wave of non-specific inflammatory cells, which will, in turn, induce tumor regression. Upon complete elimination of the tumor and the cessation of exogenous IL-2 administration, most of the effector cells should die. A small proportion of the effectors may further differentiate into memory cells, allowing surveillance and prevention of relapse.

In this scenario, many questions remain open. For example, it is not known what cell population – cytotoxic or helper CD4 lymphocytes – should be transduced to obtain the optimal anti-tumor response and how much – and for how long – IL-2 must be co-administered with the redirected T cells. In addition, the pharmacokinetic behavior and homing patterns of the redirected cells to the tumor site are yet to be studied. Properties of the targeted tumor cells must also be investigated to determine the density of tumor antigen expression needed for recognition and elimination and the fate of antigen-negative tumor variants. The system must be optimized for maximal killing of tumor cells, expressing high levels of tumor associated antigens, while sparing normal cells which may express low levels of similar surface antigens. These questions can only be addressed in an in vivo system. Because of the difficulties in expressing foreign genes in T cells, such studies are limited to activated T cell lines from specific subpopulations. The best source of various effector cells to perform in vivo studies is transgenic mice that express chimeric receptor genes. Transgenic mice, such as those described by Brocker and Karjalainen [6], should help yield answers to many of the questions posed above.

In practice, even after optimizing the conditions for therapeutic use of T-bodies, several technical problems remain, which must be solved before this approach can be tested in clinical studies. The most severe is the lack of an efficient transgene delivery and expression system in T cells. The inefficient transfection systems in use necessitate a selection step for receptor-positive transfectants, resulting in a prolonged period in culture before transfectants can be reinfused into patients. Following such prolonged culture in the presence of lymphokines, the cells take on the characteristics of lymphoblastoid cells, which, after reinfusion, are often trapped in the reticuloendothelial tissues in the lung and liver, rather than homing to the tumor site. More efficient gene delivery and expression will shorten the culture period required before reinfusion of cells. In addition, optimal culture conditions should be established, using minimal IL-2 concentrations to reduce blast transformation of the cells. In fact, since most of the anti-tumor effect of passively transferred effector lymphocytes is manifested during the initial 8–24 h following cell transfer, a transient expression system could be used. Using transient transfections, a high proportion of cells express the transfected gene and high levels of expression are usually obtained, without impairing their tumor localization.

Conclusions

Chimeric receptors containing antibody-derived Fv or scFv as their extracellular recognition elements can redirect the specificity of T cells in an MHC-independent manner. Upon encountering their target cells, such T-bodies are able to undergo specific stimulation for interleukin/cytokine production, and kill hapten-modified or tumor cells in both in vitro and in vivo model systems. T cells expressing chimeric receptors

are able to discriminate between antigen-expressing and normal cells, with negligible bystander cytotoxicity. Unlike antibodies, T cells are well suited to penetrate and destroy solid tumors. Further in vivo studies should be carried out to evaluate and optimize the persistence, homing patterns, and reactivation potential of T-bodies in vivo.

Several technical obstacles must be overcome before this approach may be applied clinically. A most urgent problem is the low efficiency of T cell transfection techniques and the particular difficulty of transducing primary T cell populations. While retroviral-mediated gene transfer is more efficient than conventional techniques such as electroporation, the proportion of transfected cells remains low, necessitating an enrichment step. In addition, antibodies with improved discrimination between cell-bound and soluble forms of tumor antigens must be obtained to expand the repertoire of tumor antigens which may be targeted. For each antigen-antibody system, the optimal design of the scFv must be determined.

In the future application of this technology, the recognition element used for chimeric TCR is not limited to antibody-derived fragments [27]. Various ligands may be coupled to a T cell-triggering molecule in an attempt to redirect cytotoxic function towards target cells expressing a particular receptor molecule. Although still experimental, we feel that with fine tuning, the T-body approach shows promise as an efficient and broad-spectrum modality for tumor immunotherapy.

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